

Serial No.: 09/674,462
Filed: May 8, 2001
Group Art Unit: 1644

On page 11, please amend the table as follows:

Domain	Human <u>(SEQ ID NO: 33)</u>	Bovine <u>(SEQ ID NO: 34)</u>
Signal peptide	76%	81.3%
Extracellular domain	85.2%	86.3%
Transmembrane domain	92.3%	96.2%
Cytoplasmic domain	96.5%	97.7%
Overall	86.1%	88.3%

On page 11, beginning at line 2, please amend the specification as follows:

Figure 4 shows the amino acid sequence of the pCTLA4-Ig construct (SEQ ID NO: 3). The underlined sequence shows the flexible linker GGSGGAA (SEQ ID NO: 28), which also denotes the junction between pCTLA4 and the IgG1 domains.

On page 11, beginning at line 13, please amend the specification as follows:

Figure 8 shows the nucleotide sequence of an anti-human CTLA-4 sFv (SEQ ID NO: 4). The inferred protein sequence is shown in **Figure 9** (SEQ ID NO: 5). **Figure 10** (SEQ ID NOS: 6-9) shows the nucleotide sequences of four anti-murine CTLA-4 sFv. The inferred protein sequences are shown in **Figure 11** (SEQ ID NOS: 10-13). The heavy and light chains are linked by a serine-glycine linker as indicated in Figures 9 and 11.[.]

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On page 11, beginning at line 21, please amend the specification as follows:

Figure 15 shows (A) the nucleotide sequence (SEQ ID NO: 14) and (B) the amino acid sequence (SEQ ID NO: 15) of human CTLA-4. The start codon is underlined. At position -21, the sequence differs from GenBank sequence L15006, and at position 110 the sequence differs from both L15006 and M74363.

On page 12, beginning at line 1, please amend the specification as follows:

Figure 16 shows the sequence of cloned human CD8 α (SEQ ID NO: 16). This differs from the GenBank sequence at positions 231 (T \rightarrow G), 244 (A \rightarrow G), 266 (T \rightarrow C), and 437 (T \rightarrow C).

On page 12, beginning at line 17, please amend the specification as follows:

Porcine CTLA-4 (“pCTLA4”) was cloned from PHA-activated pig T cells. RNA was prepared using standard techniques and pCTLA4 was amplified by PCR using primers:

5' -TTGAAGCTTAGCCATGGCTTGCTCTGGA- 3' (SEQ ID NO: 17) (5' primer)

5' -TAATGAATTCTCAATTGATGGAAATAAAATAAG -3' (SEQ ID NO: 18) (3' primer)

On page 12, beginning at line 25, please amend the specification as follows:

The predicted amino acid sequence of pCTLA4 is shown in figure 2, with a comparison with that of human and cattle. Of significance is the predicted amino acid difference at residue 97, which is important in B7 binding, being part of the conserved hexapeptide motif MYPPPY (SEQ ID NO: 29). In pCTLA4, residue 97 is leucine (giving LYPPPY (SEQ ID NO: 30)), whereas other

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species have methionine (although leucine has also been found in bovine CD28 (21)). This important amino acid difference is believed to be of key importance to the advantageous differential binding of pCTLA4 to human and pig B7.

On page 13, line 3, please amend the specification as follows (Please note that the text "TGCAGCACCACCGGAGCCACC" has not been added by way of this amendment. This text was underlined in the specification as filed and it should be underlined in the unmarked version of this paragraph.):

The extracellular domain of pCTLA4 was amplified using the 5' primer described above and:

5'-CGGTTCTGCAGCACCACCGGAGCCACCATCAGAATCTGGCATGGTCTGGAT
CAATGAC-3' (SEQ ID NO: 19)

This amplified from position 484, introduced an 18 base-pair segment encoding a linker GGSGGAA (SEQ ID NO: 28) sequence (underlined), and introduced a *Pst*I site (bold) to allow in-frame ligation to the hinge region of human IgG1. The resulting 500bp fragment was sub-cloned into *Hind*III/*Pst*I digested pBluescript-IgG1 containing genomic DNA encoding intronic sequences and the hinge, CH2, CH3 and 3' untranslated exons of human IgG1 between *Pst*I/*Not*I sites. The amino acid sequence of the resulting soluble pCTLA4-Ig is shown in figure 4.

On page 15, beginning at line 7, please amend the specification as follows (Please note that the text "GCGGCCG" and "CTGCAG" has not been added by way of this amendment. This text was underlined in the specification as filed and it should be underlined in the unmarked version of this paragraph.):

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The *myc* sequences from pHOOK1 were amplified by PCR using the 5' primer 5'-GAGCTGAAACGGGCCGCCAGAAC-3' (SEQ ID NO: 20), which contains a *NotI* site (underlined) and the 3' primer 5'-CTGGCCTGCAGCATTAGATCC-3' (SEQ ID NO: 21), which introduced a *PstI* site (underlined). The resulting 113 base pair fragment was sub-cloned into *NotI/PstI* digested pBluescript.

On page 16, beginning at line 7, please amend the specification as follows:

RNA from PHA-activated human T cells was prepared using standard techniques. hCTLA4 was amplified PCR using primers:

5'-TTCAAAGCTTCAGGATCCTGAAAGGTTTG-3' (SEQ ID NO: 22) introducing a *HindIII* site (5' primer)

5'-TAATGAATTCTCAATTGATGGAAATAAAATAAG-3' (SEQ ID NO: 23) introducing a *EcoRI* site (3' primer)

On page 16, beginning at line 15, please amend the specification as follows
(Please note that the text "ACCACCGGAGCCACC" has not been added by way of this amendment. This text was underlined in the specification as filed and it should be underlined in the unmarked version of this paragraph.):

The extracellular domain of hCTLA-4 was amplified using 5' primer described above and:

5'-GATGTAGATATCACAGGCGAAGTCGACACCACCGGAGCCACCAATTACATAA
ATCTGGGCTCCGTTGCCTATGCC-3' (SEQ ID NO: 24)

This amplified from position 457 and included a 15 base segment encoding a flexible GGSGG (SEQ ID NO: 35) amino acid linker (underlined), along with a unique *SaII* site (highlighted).

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The resulting fragment was sub cloned into *Hind*III/*Eco*RI digested pBluescript and sequenced.
hCD8 was PCR-amplified from resting T-cells using primers:

5'-TCGCGCCCAAGCTTCGAGCCAAGCAGCGT-3' (SEQ ID NO: 25) introducing a *Hind*III site (5' primer)

5'-TAATGAATTCTCAATTGATGGAAATAAAATAAG-3' (SEQ ID NO: 26) introducing an *Eco*RI site (3' primer)

On page 16, beginning at line 27, please amend the specification as follows
(Please note that the text "GGTGGCTCCGGTGGT" has not been added by way of this amendment. This text was underlined in the specification as filed and it should be underlined in the unmarked version of this paragraph.):

The transmembrane (TM) and cytoplasmic (C) domains of hCD8 were amplified using the 3' primer described above and the following 5' primer:

5'-CATAGGCAACGGAGCCCAGATTATGTAATTGGTGGCTCCGGTGGTGACT
TCGCCTGTGATATCTACATC-3' (SEQ ID NO: 27)

On page 17, beginning at line 1, please amend the specification as follows:

This amplified from position 532 and included a 15 base segment encoding a flexible GGS_n (SEQ ID NO: 35) amino acid linker (underlined), along with a unique *Sa*II site (highlighted). The resulting fragment was sub cloned into *Hind*III/*Sa*II digested pBluescript and called pBluescript-hCD8.